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Leucine-rich glioma inactivated 3: Integrative analyses reveal its potential prognostic role in cancer

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Abstract. Leucine-rich glioma inactivated 3 (LGI3) is a secreted protein in vertebrates, which belongs to the LGI family. In our previous study, LGI3 was found to be expressed in brain, adipose tissues and the skin, where it functions as a multifunctional cytokine. In the present study, we used bioinformatic tools to perform data mining, phylogenetics and prognostic association analysis to investigate the prognostic role of LGI3 in cancers. The sequences of LGI3 orthologues were analyzed from various species, and it was found that LGI3 was highly conserved in mammals and that the subsets of amino acid residues were phylogenetically coevolved in four major clusters. Single nucleotide polymorphisms (SNPs) of the human LGI3 gene included 228 functionally relevant variants (missense, nonsense and frameshift) in a total of 1,042 SNPs. Four missense SNPs had a global minor allele frequency ≥0.001. Somatic mutations in cancer with functional relevance were found in various types of cancer, including uterine, stomach and lung cancer. In addition, five amino acid residues with cancer mutations were shown to be coevolved in the vertebrate phylogeny, suggesting their importance in protein dysfunctions in cancer. One conserved amino acid and three SNPs were found to be mutated in stomach cancer and melanoma. Analysis of expression microarray data demonstrated that the expression of LGI3 was significantly associated with the prognosis of brain, colorectal and lung cancer. Taken together, these results suggested that the genetic variations and expression levels of LGI3 have potential value in cancer prognosis.

Introduction

Leucine-rich glioma inactivated 3 (LGI3) is a secreted protein member of the LGI family in vertebrates, which is expressed at high levels in the brain in a developmentally-regulated manner (1). The expression of LGI3 in the brain has been shown to be regulated at the transcriptional level by activating protein-2 and neuronal restrictive silencer (1). In our previous studies, it was reported that LGI3 regulated neuronal exocytosis and differentiation (2,3). In addition to the nervous system, LGI3 is expressed in various tissues, including adipose tissues and the skin (4,5). Our previous study demonstrated that the ultraviolet B-irradiation-induced secretion of LGI3 from human keratinocytes protected cells (5), and it was further shown that LGI3 promoted the migration of keratinocytes and melanogenic pigmentation (6,7).

Our previous studies also showed that the expression of LGI3 was downregulated during adipocyte differentiation and was upregulated in the adipose tissues of ob/ob mice and high fat diet-fed obese mice (4,8). It was shown that LGI3 attenuated adipogenesis through its receptor, a disintegrin and metalloproteinase domain-containing protein 23 (ADAM23), and that LGI3 increased the expression of inflammatory proteins, including tumor necrosis factor-α (TNF-α) in macrophages (4). LGI3 was shown to downregulate adiponectin, an anti-inflammatory adipokine (8). LGI3 and TNF-α were also found to be upregulated mutually through nuclear factor-κB (NF-κB), suggesting their importance in metabolic inflammation in obesity (9). Therefore, it was hypothesized that LGI3 is involved as a pro-inflammatory cytokine, which interacts with TNF-α and adiponectin, and these results supported the hypothesis that LGI3 is a multifunctional cytokine secreted by, and acting at, multiple cell types (3-9).

Cytokines, including TNF-α and adiponectin have been described as risk factors, and potential diagnostic and prognostic biomarkers in cancer (10,11). As LGI3 has been shown to interact with TNF-α and adiponectin in metabolic inflammation (8,9), it was hypothesized that LGI3 may also be associated with the cytokine network in cancer. To confirm this hypothesis, the present study analyzed the phylogeny of LGI3 orthologues, amino acid coevolution, single nucleotide polymorphisms (SNPs), somatic mutations and expression microarray data in different types of cancer. The results of these integrative analyses supported the potential significance of LGI3 in cancer prognosis.

Materials and methods

Sequence retrieval and phylogenetic analysis. All the LGI3 genes and amino acid sequences were obtained from the

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Ensembl database (http://www.ensembl.org). Comparative sequence analysis and alignment were performed using Blastp in NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE=Proteins) and ClustalW algorithm implemented in MEGA 6.0 software (http://www.megasoftware.net). A maximum likelihood tree of LGI3 was constructed using MEGA 6.0 with the Kimura 2 parameter model. Coevolution analysis of amino acid sequences was performed using Coevolution Analysis of Protein Sequences (CAPS 2.0; http://bioinf.gen.tcd.ie/caps) (12). The coevolved amino acid clusters were selected and diagrams were constructed using the Cytoscape 3.4.0 program (http://www.cytoscape.org).

SNP data evaluation. The SNPs of human LGI3 (GenBank accession no.: AAM49554.1) were extracted from the Ensembl (http://www.ensembl.org) and NCBI (http://www.ncbi.nlm.nih.gov) SNP databases. Functionally relevant SNPs, which disrupted reference amino acid sequence (missense, nonsense, frameshift and splice site variants) were selected for comparative analysis. SNPs with known global minor allele frequency (MAF) values were taken into account.

Somatic mutations in cancer. Somatic mutations of the human LGI3 gene in cancer were identified in Cbioportal (http://www.cbioportal.org) (13,14), the Catalogue of Somatic Mutations in Cancer (COSMIC; https://cancer.sanger.ac.uk/cosmic) and The Cancer Genome Atlas (TCGA; https://tcga-data.nci.nih.gov/tcga). A Venn diagram of the categorized genetic variations was generated using Venny 2.0.2 (http://bioinfogp.cnb.csic.es/tools/venny/index.html). The expression data of LGI3 in normal and cancer tissues were obtained from the Human Protein Atlas (http://www.proteinatlas.org) and NCBI UniGene (http://www.ncbi.nlm.nih.gov/unigene/).

Meta-analysis of expression microarray data. Gene expression microarray datasets were searched using the PrognoScan database (http://www.prognoscan.org) (15). This database consists of a large collection of publicly available cancer microarray datasets, providing sample name, raw expression data file, sample source name, array platform, and clinical annotations, including tumor grade diagnosis, histological diagnosis, age at diagnosis, survival time, treatment and therapy types. These datasets are previously subjected to quality control tests, normalization and batch effect adjustment, with exclusion of low-quality samples. The assessment of associations between gene expression and cancer prognosis use the minimum P-value approach for grouping patients for survival analysis, which identifies the optimal cutoff point in continuous gene expression measurement without prior assumption. Briefly, the patients ordered by the expression values were dichotomized at the cutoff point to minimize the P-value, and the survival difference between the high and low expression groups were calculated using the log-rank test. Kaplan-Meier plots of statistically significant ($P<0.05$; group size >10) datasets were generated.

Results

Phylogenetic analysis of LGI3 protein. As the LGI3 gene was initially identified in humans and mice (1,16), LGI3 gene orthologues were found only in vertebrates. The sequences of LGI3 gene products were retrieved from the Ensembl database and confirmed using BLASTp in the NCBI database. The complete LGI3 gene products were identified in 42 species, which belonged to vertebrates (phylum Chordata; subphylum Vertebrata). The phylogenetic tree was constructed according to the protein sequences of LGI3 (Fig. 1). The LGI3 protein from the Mammalia class (mammals) formed a highly conserved cluster (Fig. 1). A total of 38 amino acids residues of the LGI3 protein (548 amino acids) were identical in all species analyzed (Fig. 2). These conserved amino acids were distributed throughout all LGI3 protein domains, with the exception of the amino terminal region, including the first and second leucine-rich repeats (LRRs) and the carboxy terminal seventh epitempin (EPTP) domain.

Analysis of the phylogenetic tree of the LGI3 protein sequences may provide insight into the functionally important residues and their variants in diseases. Coevolution analysis was performed using the human LGI3 protein sequence as an input and 55 orthologues, including 42 full-length sequences, in CAPS software, which identifies coevolution between amino acid residues. The results showed that 100 amino acid residues were involved in coevolved amino acid pairs (Fig. 3A). The distribution of coevolution degrees revealed four clusters with a high coevolution degree: Cluster a, Thr70-Leu93; cluster b, Phe132-Asp164; cluster c, Val281-Leu299; and cluster d, Ala369-Leu449 (Fig. 3B). The amino acids in cluster b appeared to be highly coevolved, compared with those in the other clusters. Cluster b corresponded predominantly to the fourth LRR domain (LRR4; Fig. 2).

SNPs of the human LGI3 gene. A total of 1,042 SNPs were identified in the human LGI3 gene from the Ensembl and NCBI SNP databases. The SNPs which caused protein sequence variations were collected for comparative analysis. These included 217 missense SNPs, five nonsense SNPs and six frameshift SNPs. Of the 42 missense SNPs with known global MAF, four SNPs had a global MAF of ≥0.001 (Table I). These SNPs were distributed in the entire protein region, with the exception of the LRR2 domain (residues 88-113). These SNPs included a conserved residue (Gly466; Figs. 2 and 4) and three residues (Asn73, Gln204 and Tyr241) shown to be coevolved in the vertebrate phylogeny (Figs. 3 and 4).

Somatic mutations of LGI3 in cancer. The search for somatic mutations of the human LGI3 gene with amino acid alterations was performed using the Cbioportal, COSMIC and TCGA public databases. Mutations were found in various types of cancer, including uterine, stomach, lung, head and neck, skin, liver and bladder cancer (Table II). Venn diagram analysis of the amino acid variations in the four categories (conserved residues, coevolved residues, SNPs and somatic mutations in cancer) showed that a subgroup of somatic mutation sites in cancer belonged to conserved residues (one residue), coevolved residues (five residues) and SNPs (three residues), as shown in Fig. 4. The conserved residue (Gln319) was mutated in stomach cancer (Fig. 4; Table IIIA). The five coevolved amino acids were found to be mutated in liver, uterine, bladder, lung and thyroid cancer (Fig. 4; Table IIIB). The three SNP minor

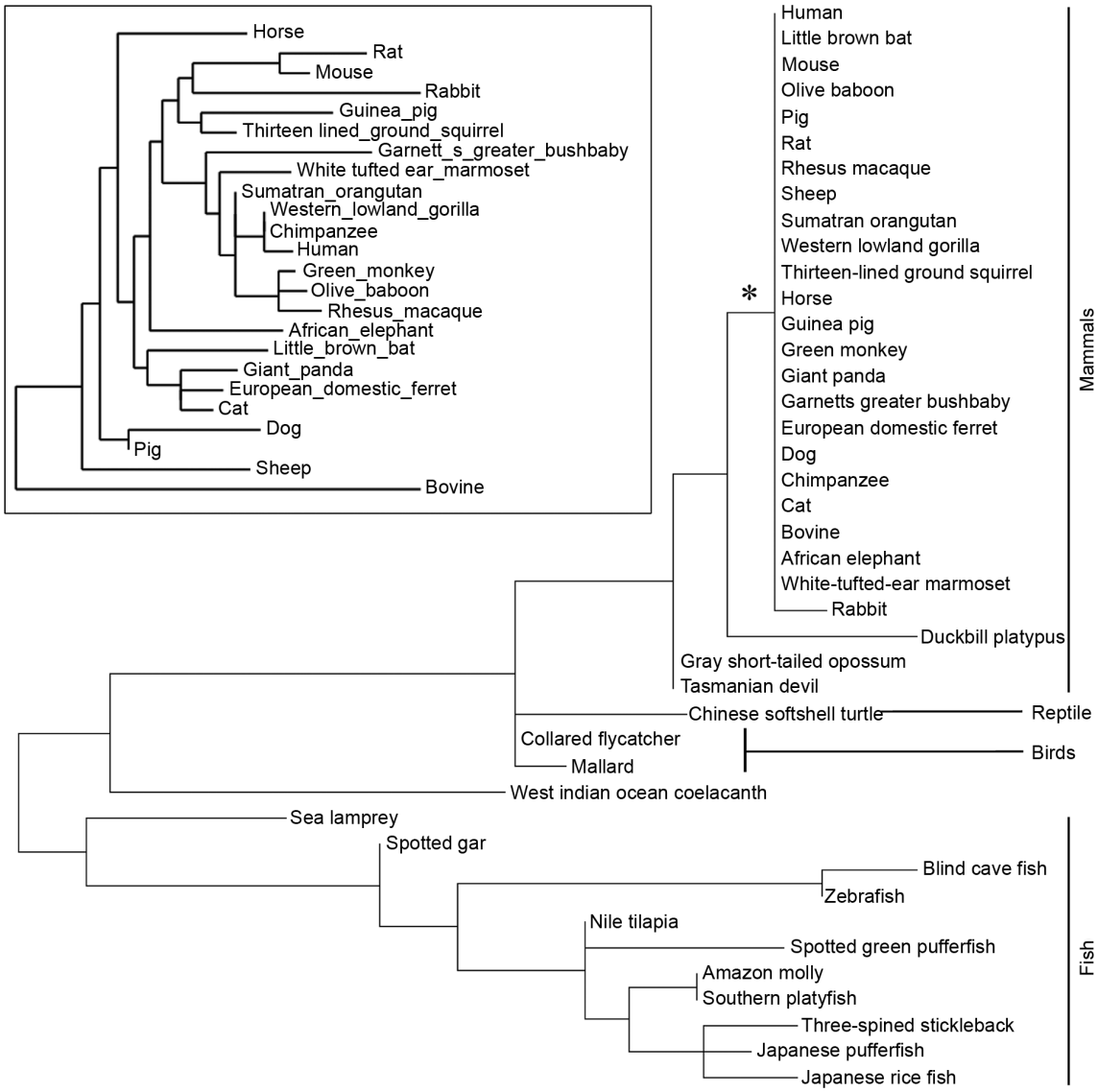

Figure 1. Phylogenetic tree of LGI3. The diagram was generated according to the amino acid sequences of LGI3 orthologues. The boxed diagram represents an expanded tree of mammals (marked* class Mammalia). LGI3, leucine-rich glioma inactivated 3.

alleles were found in somatic mutations of stomach cancer and melanoma (Fig. 4; Table IIIC).

Prognostic significance of the expression of LGI3 in cancer. A previous study reported that LGI3 is expressed in various tumor cell lines (17). To examine the significance of the expression of LGI3 in the prognosis of cancer, the gene expression microarray datasets of cancer patient cohorts were analyzed. The results revealed an association between the expression level of LGI3 and cancer prognosis in brain cancer (astrocytoma), colorectal cancer and non-small cell lung cancer (Fig. 5A-C; Table IV). In these types of cancer cohorts (18-21), a lower expression of LGI3 was significantly correlated with poor patient survival rates (Fig. 5).

Discussion

LGI3 is a member of the LGI protein family, which consists of four secreted proteins (LGI1, 2, 3 and 4) (1,22). Protein members of the LGI family are distinct in tissue distribution and physiological function (22,23). Our previous studies supported the roles of LGI3 as a cytokine in adipose tissues and the skin (4-9). The founding member of the LGI family, LGI1, was shown to be deficient in malignant gliomas due to gene rearrangements and was suggested to be a tumor suppressor gene (24). LGI protein members have been shown to be differentially expressed in various tumor cell types, including glioma, neuroblastoma, melanoma, colon cancer and breast cancer (17). These results indicated genetic alterations of these genes during tumorigenesis. To examine the association between LGI3 and cancer, the present study performed integrative analyses using phylogenetic and coevolution analyses, combined with the analyses of genomic variations and expression array data.

The phylogenetic tree of the LGI3 protein was restricted to vertebrates and formed the class-specific clusters of mammals, bird, reptiles and fish (Fig. 1). In particular, the highly conserved mammalian cluster of LGI3 sequences supports the validity of mammalian model systems in investigations of associations between cancer and LGI3 sequence variations.

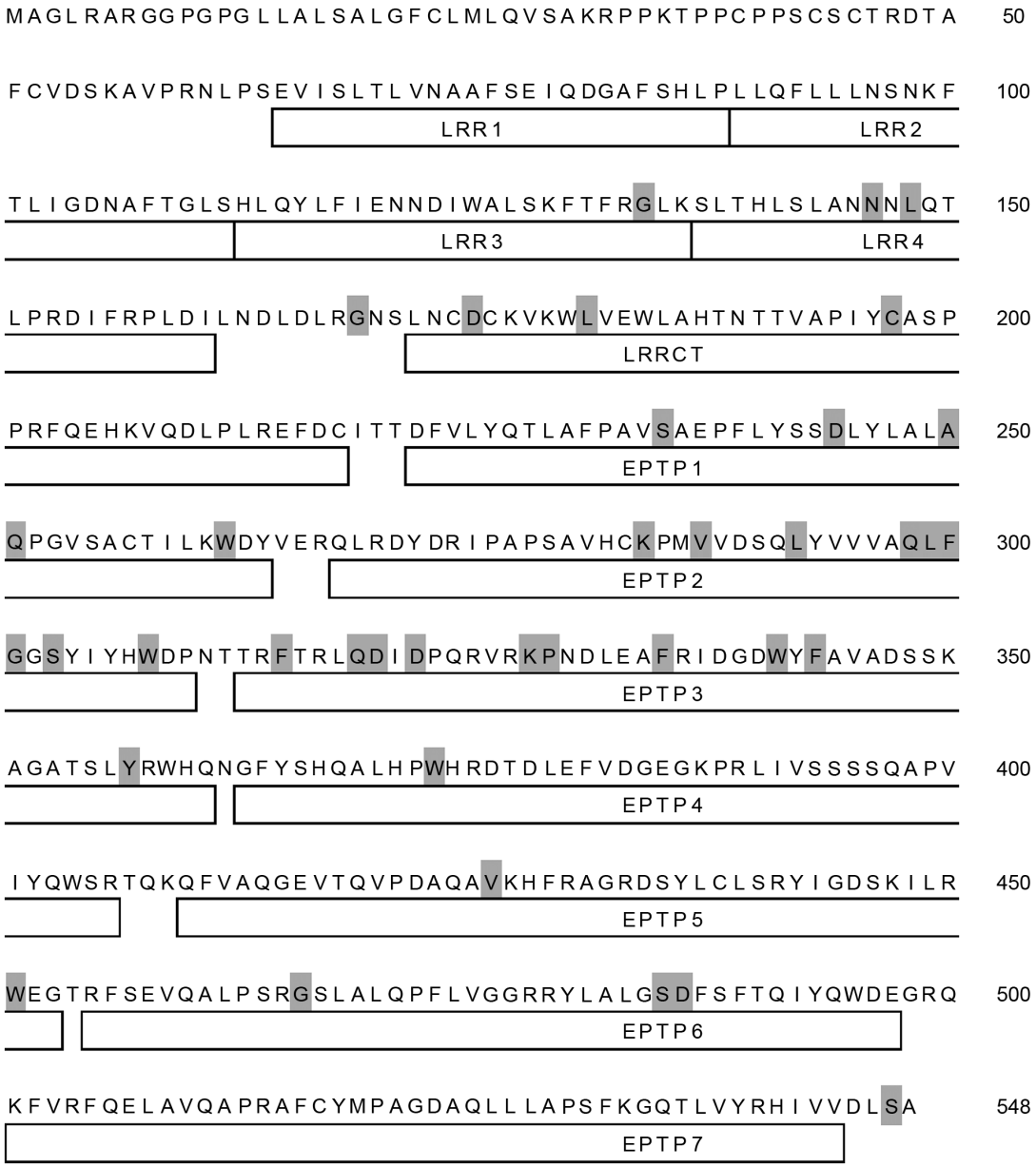

Figure 2. Sequence and domain structure of the human leucine-rich glioma inactivated 3 protein. Conserved amino acids in all available orthologue sequences are shaded. Tandem repeats of LRR and EPTP are numbered. LRR, leucine-rich repeat; EPTP, epitempin.

Conserved amino acid residues were relatively frequent in the EPTP2 and EPTP3 domains, suggesting their structural and functional importance (Fig. 2). Coevolution analysis revealed clusters with a high degree of coevolution of amino acid residues (Fig. 3). These conserved or coevolved residues may serve as crucial amino acids for the biologically active, native protein structure. Conserved residues are often found in global hinges in proteins, whereas coevolution propensity is associated with substrate or ligand recognition sites (25). Although the structure and ligand interactions of LGI3 protein remain to be fully elucidated, genetic variations of the conserved and coevolved residues are predicted to perturb its normal structure and function. Of 42 functionally relevant SNPs with a known global MAF (Table I), one SNP residue (Gly466) at a conserved residue and three SNPs (Asn73, Gln204 and Tyr241) at coevolved residues were predicted to affect the structure and function of LGI3.

The comparative analysis of somatic mutations in cancer, amino acid sequence variations and coevolution revealed eight somatic mutations, which occurred at coevolved or polymorphic residues (Fig. 4). These variants were found in various types of cancer (Table IIIB and C). All somatic mutations at coevolved amino acids located in LRR domains, which are predicted to be involved in protein-protein interactions for homo- or heterodimerization (22,26). As coevolved amino acids have been implicated in intramolecular interactions and protein network formation (12,27), functional investigations on variations of these amino acids may provide insight into the involvement of LGI3 in tumorigenesis. All SNPs with somatic mutations in stomach cancer and melanoma were rare variants (global MAF <0.001). Rare SNPs are often associated with cancer predisposition (28,29).

The expression of LGI3 is widespread in various tissues, including the brain, lung, skin, adipose tissues, heart, placenta,

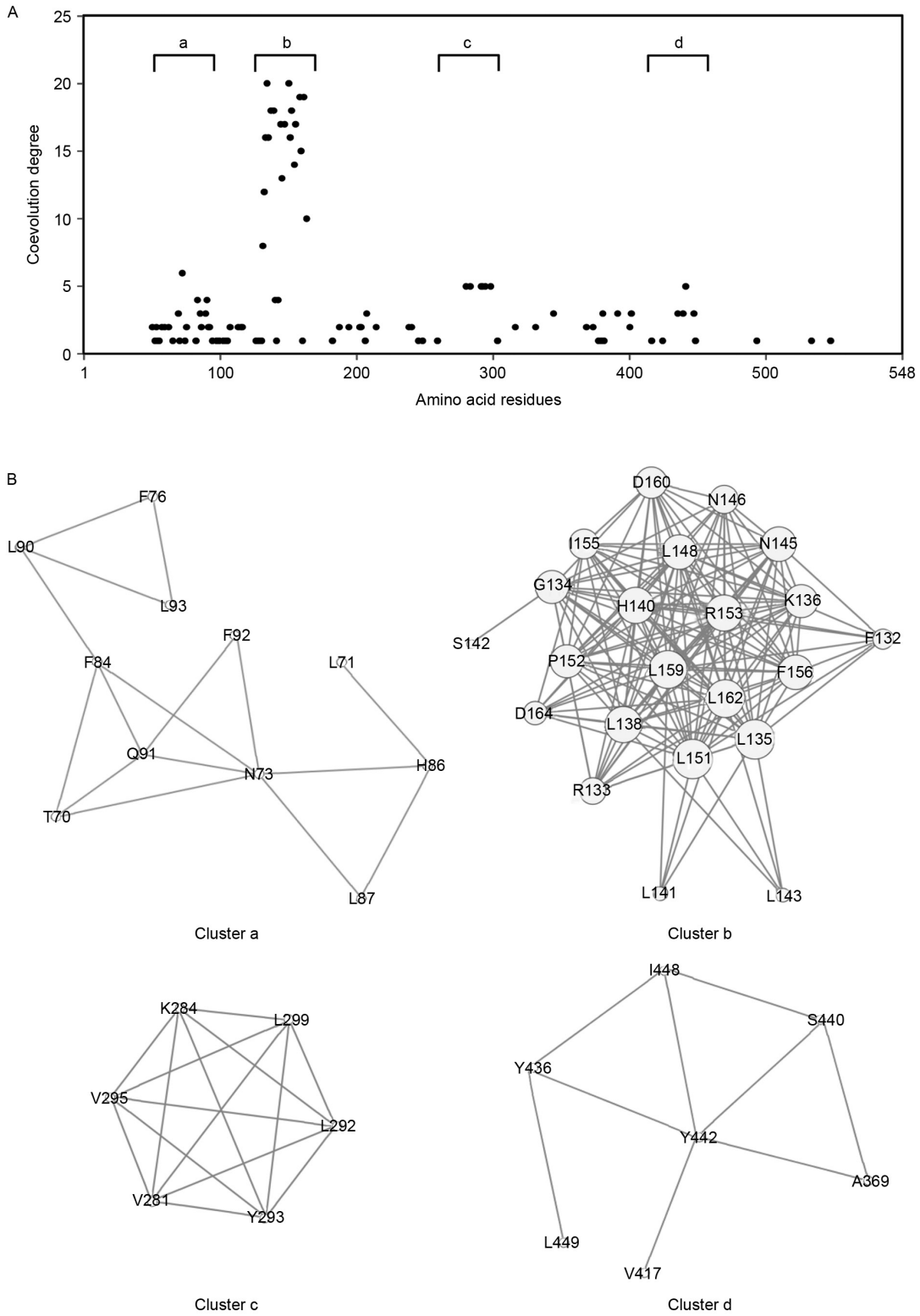

Figure 3. Coevolution of amino acid residues of the leucine-rich glioma inactivated 3 protein. (A) Distribution of coevolved amino acids in protein sequence. Amino acid clusters (a, b, c and d) with a high coevolution degree are indicated. (B) Coevolution diagram. Coevolved amino acid pairs are indicated as circles (amino acid residues) and lines (coevolved pair). The size of the circle is proportional to the coevolution degree of the amino acid residue. In total, 16 clusters of coevolution were detected with 54 amino acids involved. Four amino acids clusters with a maximum coevolution degree (≥5) are indicated.

liver, muscle, kidney and pancreas (1,4,5,16). Predominant expression and its functional elucidation has been reported in the brain, adipose tissue and skin (3-5,9). A previous report showed that LGI3 was expressed in glioma, neuroblastoma, melanoma, colon cancer and breast cancer cells (17). Among four LGI family members, LGI3 was the only member expressed significantly in gliomas, melanomas and neuroblastoma (17). The present study found that the expression of LGI3 was associated with the prognosis of brain, colorectal and lung cancer (Fig. 5). The expression levels of LGI3 in

Table I. Functionally relevant SNPs of the human leucine-rich glioma inactivated 3 gene.

Chromosome 8 position	ID	Type	SNP allele	SNP amino acid	Residue number	Global MAF
22148237	rs34112456	Missense	A	Thr (T)	524	0.0214
22156509	rs571516031	Missense	T	Ser (S)	12	0.0170
22148657	rs149352514	Missense	A	Ser (S)	384	0.0084
22151884	rs199663838	Missense	G	Arg (R)	204	0.0020
22148609	rs150255699	Missense	A	Ile (I)	400	0.0008
22154149	rs146853993	Missense	G	Ala (A)	139	0.0008
22148200	rs150789268	Missense	T	Met (M)	536	0.0006
22148372	rs149918878	Missense	T	Cys (C)	479	0.0006
22148509	rs113893603	Missense	A	His (H)	433	0.0006
22148341	rs562289764	Missense	C	Ser (S)	489	0.0004
22151597	rs377407416	Missense	C	His (H)	241	0.0004
22151854	rs571880878	Missense	A	Gln (Q)	214	0.0004
22151891	rs569108469	Missense	T	Cys (C)	202	0.0004
22148186	rs573206061	Missense	T	Tyr (Y)	541	0.0002
22148236	rs202037316	Missense	T	Val (V)	524	0.0002
22148333	rs201040656	Missense	G	Val (V)	492	0.0002
22148410	rs541459476	Missense	A	Asp (D)	466	0.0002
22148426	rs559732127	Missense	A	Thr (T)	461	0.0002
22148437	rs145000513	Missense	T	Leu (L)	457	0.0002
22148443	rs115515473	Missense	A	His (H)	455	0.0002
22148519	rs531970563	Missense	T	Cys (C)	430	0.0002
22148590	rs568594233	Missense	A	His (H)	406	0.0002
22148622	rs200322572	Missense	G	Arg (R)	395	0.0002
22148827	rs143158388	Missense	A	His (H)	327	0.0002
22148833	rs370352885	Missense	A	His (H)	325	0.0002
22148869	rs559672591	Missense	G	Arg (R)	313	0.0002
22148872	rs572016004	Missense	T	Ile (I)	312	0.0002
22151503	rs201436266	Missense	G	Cys (C)	272	0.0002
22151519	rs560898710	Missense	T	Trp (W)	267	0.0002
22151903	rs138152858	Missense	A	Thr (T)	198	0.0002
22151983	rs374479882	Missense	T	Leu (L)	171	0.0002
22151993	rs371552621	Missense	T	Trp (W)	168	0.0002
22153990	rs550303759	Missense	G	Ala (A)	158	0.0002
22154013	rs568454198	Missense	T	Ile (I)	150	0.0002
22154155	rs555889097	Missense	C	Pro (P)	137	0.0002
22154199	rs184939949	Missense	G	Ser (S)	122	0.0002
22155450	rs199694884	Missense	A	Thr (T)	74	0.0002
22155453	rs544969538	Missense	T	Tyr (Y)	73	0.0002
22156358	rs530550129	Missense	G	Arg (R)	62	0.0002
22156430	rs200873593	Missense	G	Arg (R)	38	0.0002
22156469	rs190789584	Missense	C	Thr (T)	25	0.0002
22156517	rs538737490	Missense	A	Asp (D)	9	0.0002

SNP, single nucleotide polymorphism; MAF, minor allele frequency.

these types of cancer were positively correlated with survival rates. This suggested that LGI3 may function as a suppressor of tumor progression. Somatic mutations and associations between expression and prognosis were found in lung cancer (Tables IIIB and IV). LGI3 was shown to transduce signals in its target cells through proteins implicated in cancer, including ADAM23, Akt, β-catenin, focal adhesion kinase, MDM2, p53, NF-κB and microphthalmia-associated transcription factor (3,5,6,9,30-33). Perturbation of these proteins by somatic mutations and the altered expression of LGI3 may

Table II. Somatic mutations of leucine-rich glioma inactivated 3 in cancer tissues.

Cancer study	Sample ID	Amino acid change	Type	VAF (Normal)	VAF (Tumor)
Uterine (TCGA pub)	TCGA-D1-A17F-01	X277_splice	Splice	NA	0.42
Uterine (TCGA pub)	TCGA-AP-A056-01	G302S	Missense	NA	0.41
Uterine (TCGA pub)	TCGA-B5-A0JY-01	F223V	Missense	NA	0.37
Uterine (TCGA pub)	TCGA-D1-A103-01	S242F	Missense	NA	0.37
Uterine (TCGA pub)	TCGA-D1-A163-01	R433C	Missense	NA	0.37
Uterine (TCGA pub)	TCGA-BG-A0M2-01	K447R	Missense	NA	0.32
Uterine (TCGA pub)	TCGA-E6-A1LZ-01	E237*	Nonsense	NA	0.27
Uterine (TCGA pub)	TCGA-AX-A05Z-01	F92L	Missense	NA	0.24
Uterine (TCGA pub)	TCGA-B5-A0JY-01	D210A	Missense	NA	0.22
Uterine (TCGA pub)	TCGA-B5-A11E-01	S349F	Missense	NA	0.17
Uterine (TCGA pub)	TCGA-AP-A0LM-01	D331N	Missense	NA	0.12
Uterine (TCGA pub)	TCGA-AP-A0LM-01	A482T	Missense	NA	0.11
Uterine (TCGA)	TCGA-D1-A17F-01	X277_splice	Splice	NA	0.42
Uterine (TCGA)	TCGA-AP-A056-01	G302S	Missense	NA	0.41
Uterine (TCGA)	TCGA-B5-A0JY-01	F223V	Missense	NA	0.37
Uterine (TCGA)	TCGA-D1-A103-01	S242F	Missense	NA	0.37
Uterine (TCGA)	TCGA-D1-A163-01	R433C	Missense	NA	0.37
Uterine (TCGA)	TCGA-BG-A0M2-01	K447R	Missense	NA	0.32
Uterine (TCGA)	TCGA-E6-A1LZ-01	E237*	Nonsense	NA	0.27
Uterine (TCGA)	TCGA-AX-A05Z-01	F92L	Missense	NA	0.24
Uterine (TCGA)	TCGA-B5-A0JY-01	D210A	Missense	NA	0.22
Uterine (TCGA)	TCGA-B5-A11E-01	S349F	Missense	NA	0.17
Uterine (TCGA)	TCGA-AP-A0LM-01	D331N	Missense	NA	0.12
Uterine (TCGA)	TCGA-AP-A0LM-01	A482T	Missense	NA	0.11
Stomach (Pfizer UHK)	pfg072T	L159Wfs[a]4	FS del	NA	0.31
Stomach (TCGA pub)	TCGA-FP-7829-01	Q491H	Missense	NA	0.53
Stomach (TCGA pub)	TCGA-BR-8680-01	T312A	Missense	NA	0.50
Stomach (TCGA pub)	TCGA-CD-A4MJ-01	G302S	Missense	NA	0.38
Stomach (TCGA pub)	TCGA-BR-A4QL-01	R327H	Missense	NA	0.33
Stomach (TCGA pub)	TCGA-HU-A4H3-01	Q491R	Missense	NA	0.22
Stomach (TCGA pub)	TCGA-BR-6452-01	R433H	Missense	NA	0.19
Stomach (TCGA pub)	TCGA-B7-5816-01	R375H	Missense	NA	0.16
Stomach (TCGA pub)	TCGA-CG-4437-01	R327H	Missense	NA	0.15
Stomach (UTokyo)	GC_313T-GC_313N	Q319K	Missense	NA	0.12
Lung adenocarcinoma (TCGA pub)	TCGA-44-2656-01	E507*	Nonsense	NA	0.19
Lung adeno (TCGA pub)	TCGA-91-6829-01	Q460L	Missense	NA	0.16
Lung adeno (TCGA)	TCGA-44-2656-01	E507[a]	Nonsense	NA	0.19
Lung adeno (TCGA)	TCGA-91-6829-01	Q460L	Missense	NA	0.16
Lung squ (TCGA pub)	TCGA-66-2795-01	L117F	Missense	NA	0.47
Lung squ (TCGA pub)	TCGA-66-2785-01	R430G	Missense	NA	0.25
Lung squ (TCGA)	TCGA-66-2795-01	L117F	Missense	NA	0.47
Lung squ (TCGA)	TCGA-66-2785-01	R430G	Missense	NA	0.25
Head and neck (Broad)	HN_62854	R514W	Missense	NA	0.19
Head and neck (TCGA pub)	TCGA-DQ-7588-01	K56Rfs[a]13	FS del	NA	0.26
Head and neck (TCGA pub)	TCGA-CQ-6228-01	P276T	Missense	NA	0.05
Head and neck (TCGA)	TCGA-DQ-7588-01	K56Rfs[a]13	FS del	NA	0.26
Head and neck (TCGA)	TCGA-CQ-6228-01	P276T	Missense	NA	0.05
Melanoma (TCGA)	TCGA-ER-A42K-06	T46I	Missense	NA	0.88
Melanoma (TCGA)	TCGA-FW-A3R5-06	R430C	Missense	NA	0.31
Melanoma (TCGA)	TCGA-FW-A3R5-06	R406C	Missense	NA	0.26

Table II. Continued.

Cancer study	Sample ID	Amino acid change	Type	VAF (Normal)	VAF (Tumor)
Melanoma (TCGA)	TCGA-FS-A4FC-06	R430C	Missense	NA	0.22
Liver (AMC)	H060607	Y539N	Missense	NA	0.27
Liver (TCGA)	TCGA-ED-A4XI-01	G302D	Missense	NA	0.18
Liver (TCGA)	TCGA-ES-A2HS-01	Q91R	Missense	NA	0.13
Bladder (TCGA)	TCGA-FD-A3SM-01	Q525H	Missense	NA	0.47
Bladder (TCGA)	TCGA-G2-A2EO-01	D160H	Missense	NA	0.13
Breast (TCGA 2015)	TCGA-AN-A0FJ-01	D320E	Missense	NA	0.34
Breast (TCGA 2015)	TCGA-D8-A1XQ-01	A351V	Missense	NA	0.11
Thyroid (TCGA pub)	TCGA-EL-A3N3-01	E215G	Missense	NA	0.28
Thyroid (TCGA)	TCGA-EL-A3N3-01	E215G	Missense	NA	0.28
DLBC (TCGA)	TCGA-G8-6324-01	A461T	Missense	NA	0.46
DLBC (TCGA)	TCGA-FF-8046-01	R514Q	Missense	0.02	0.17
Prostate (SU2C)	SC_9097	R314H	Missense	NA	0.89
chRCC (TCGA)	TCGA-KN-8428-01	A107T	Missense	NA	0.13

VAF, variant allele frequency; fs, frameshift; [a], stop; del, deletion; adeno, adenoma; squ, squamous carcinoma; DLBC, diffuse large B-cell lymphoma; chRCC, chromophobe renal cell carcinoma; NA, not applicable.

Table III. Somatic mutations of leucine-rich glioma inactivated 3 in cancer tissues.

A, Conserved residues

Amino acid change	Sample ID	Tissue	VAF
Q319K	GC_313T-GC_313N	Stomach	0.12

B, Coevolved residues

Amino acid change	Coevolution cluster	Sample ID	Tissue	VAF
Q91R	Cluster a	TCGA-ES-A2HS-01	Liver	0.13
F92L	Cluster a	TCGA-AX-A05Z-01	Uterine	0.24
D160H	Cluster b	TCGA-G2-A2EO-01	Bladder	0.13
L117F	F108, Y116	TCGA-66-2795-01	Lung	0.47
E215G	T188, F203	TCGA-EL-A3N3-01	Thyroid	0.28

C, SNPs

Amino acid change	SNP ID	Global MAF	Sample ID	Tissue	VAF
R327H	rs143158388	0.0002	TCGA-BR-A4QL-01	Stomach	0.33
R327H	rs143158388	0.0002	TCGA-CG-4437-01	Stomach	0.15
R433H	rs113893603	0.0006	TCGA-BR-6452-01	Stomach	0.19
R430C	rs531970563	0.0002	TCGA-FW-A3R5-06	Melanoma	0.31
R430C	rs531970563	0.0002	TCGA-FW-A4FC-06	Melanoma	0.22

SNP, single nucleotide polymorphism; MAF, minor allele frequency; VAF, variant allele frequency.

account for its role in the pathogenesis and prognosis of cancer. These results warrant further investigations on the correlation between LGI3 sequence variations, and the expression and multiple clinical parameters of cancer.

Table IV. Dataset description of somatic associations between expression microarray analyses of leucine-rich glioma inactivated 3 and cancer prognosis.

Dataset	Cancer	Subtype	Patients (n)	Cutoff point	P-value
GSE4271-GPL97	Brain	Astrocytoma	77	0.77	0.0457
GSE17536	Colorectal		177	0.14	0.0007
GSE3141	Lung	NSCLC	111	0.47	0.0147

NSCLC, non-small cell lung cancer.

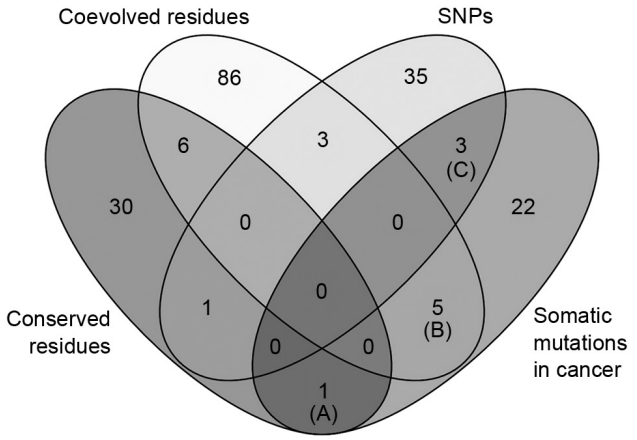

Figure 4. Comparative analysis of genetic variations and cancer somatic mutations of leucine-rich glioma inactivated 3. Venn diagram indicating amino acid distributions in the categories of variation. (A) Somatic mutations in cancer at conserved amino acids. (B) Somatic mutations in cancer at coevolved amino acids. (C) Somatic mutations in cancer at SNPs; SNPs, single nucleotide polymorphisms.

Cytokine networks have important regulatory roles in cancer and numerous cytokines are involved in inflammatory immune responses to tumors (11,34). Cytokines are important in chronic inflammation, which may lead to carcinogenesis (34). Obesity increases cancer risk through cytokine perturbations by metabolic inflammation, particularly in the liver, pancreas and gastrointestinal tract (35). TNF-α and adiponectin have been shown to be regulated by LGI3 in adipose tissues in obesity (8,9) and associated with various types of cancer (10,11). Diet-induced obesity has also been shown to elevate TNF-α and local inflammation, which may promote colorectal cancer and hepatocellular carcinoma (36). Adiponectin has been shown to be inversely correlated with the risk of endometrial, breast, colon, renal, gastric and prostate cancer (10). In conclusion, LGI3 may be involved in the cytokine network involved in various types of cancer and have a potential role in cancer prognosis.

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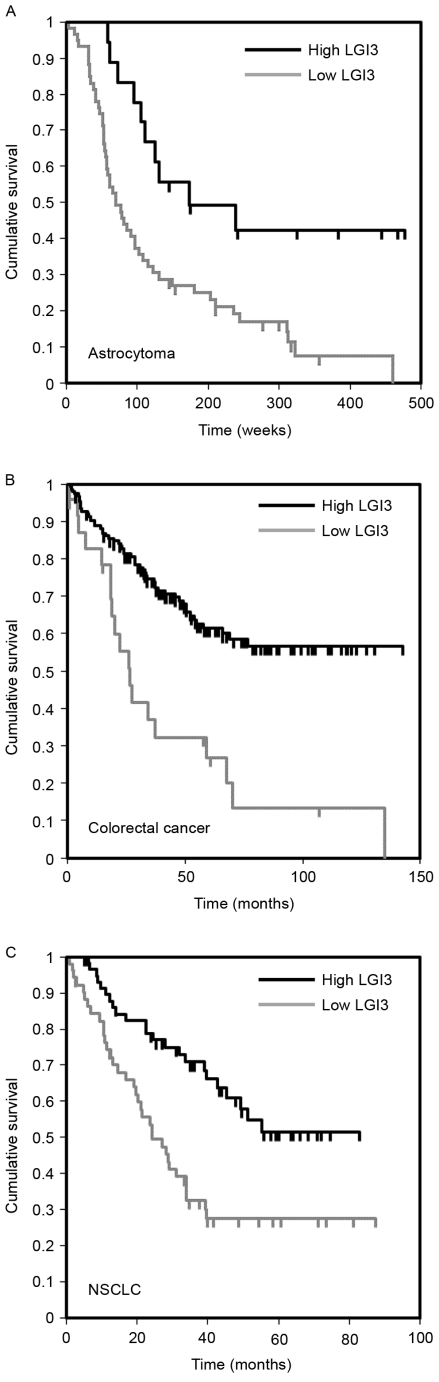

Figure 5. Associations between the expression microarray analyses of LGI3 and (A) astrocytoma, (B) colorectal cancer and (C) NSCLC prognosis. Kaplan-Meier survival curves of the microarray dataset of patient groups are shown. LGI3, leucine-rich glioma inactivated 3; NSCLC, non-small cell lung cancer.

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